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Characterization of primary and secondary polycythemia among Palestinian blood donors in the West bank

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Polycythemia is a hematological condition characterized by an elevated red blood cell (RBC) mass that exceeds the normal range for a specific age and gender; it can be classified as either primary or secondary. This study aimed to estimate the prevalence of primary and secondary polycythemia among adult blood donors in the West Bank and characterize potential risk factors. This cross-sectional study included 674 adult Palestinian male blood donors, aged 18 to 62 years, at An-Najah National University Hospital. Data were collected through face-to-face interviews. Eligible donors underwent complete blood count (CBC) analysis, and samples meeting the latest World Health Organization (WHO) diagnostic criteria for polycythemia, hemoglobin > 16.5 g/dL or hematocrit > 49% in men, were sent for DNA sequencing. Among the participants, 16.6% met the diagnostic criteria for polycythemia; however, none had the *JAK2* p.V617F mutation, indicating a potential lack of polycythemia vera (PV). Risk factor analysis revealed an increased risk of polycythemia in moderate ($p = 0.039$, OR 2.223, [95% CI 1.041–4.746]), heavy cigarette smoking ($p = 0.008$, OR 2.301, [95% CI 1.246–4.249]), and heavy waterpipe smoking ($p \leq 0.001$, OR: 5.019, [95% CI 2.364–10.653]), whereas heavy coffee consumption had a protective effect against polycythemia ($p = 0.013$, OR 0.444 [95% CI 0.234–0.843]). The absence of the *JAK2* p.V617F mutation among participants suggests that secondary polycythemia predominance is driven by smoking and other modifiable lifestyle factors. These findings emphasize the importance of public health efforts focused on lifestyle modification to reduce the risk of polycythemia.

Keywords Polycythemia, *JAK2* mutation, Blood donation, Hemoglobin, Tobacco smoking, West bank

Polycythemia is a hematological condition characterized by an elevated red blood cell (RBC) mass, indicated by increased hemoglobin (Hb) levels or hematocrit (HCT), beyond the normal range for a specific age and sex[1]. Polycythemia can be either primary or secondary on the basis of pathophysiology[2]. Primary polycythemia, also called polycythemia vera (PV), belongs to the family of classic myeloproliferative neoplasms[3]. It results from an acquired mutation in the Janus kinase 2 (*JAK2*) gene[4]. The main abnormality in almost 98% of PV cases is the V617F genetic mutation in exon 14 of the *JAK2* tyrosine kinase (source: p.V617F)[5]. On the other hand, secondary polycythemia is associated with tissue hypoxia or inappropriate secretion of erythropoietin[6]. It is more likely to occur in those who smoke, have chronic lung conditions, or reside at high altitudes[7].

Although primary and secondary polycythemia have been the subject of much research, only a few studies have highlighted their prevalence. A study conducted in the United States estimated that 22 per 100,000 people have polycythemia[8]. Another study conducted in Mosul reported a prevalence of 40 per 100,000 among the general population and 1,650 per 100,000 among blood donors[2].

There is limited research on the prevalence of JAK2 mutations in patients with polycythemia in Palestine. Only one study has explored this issue: a 2021 case-control study in the West Bank that involved three groups[9]. The study examined the most common mutation, JAK2 p.V617F, alongside the JAK2 exon 12 mutation. No

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instances of JAK2 exon 12 mutations were found, whereas JAK2 p.V617F mutations were identified across all three groups of participants[9]. Furthermore, research in the Arab region has focused on Sudanese individuals with polycythemia vera (PV). In this context, the overall prevalence of the two JAK2 mutations among Sudanese PV patients was reported to be 94.6%, with JAK2 p.V617F identified in 91% of cases and JAK2 exon 12 mutations identified in 8.1%[10].

In Palestine, evidence regarding the prevalence of polycythemia and associated risk factors is limited. Distinguishing between primary and secondary polycythemia is crucial, as it has significant management implications. Patients with polycythemia may complain of headaches, exhaustion, vertigo, itching, or thrombotic events[11]. Polycythemia, regardless of the cause, carries a risk of blood clotting due to increased viscosity, which may lead to deep vein thrombosis, stroke, and acute ischemic heart disease[12]. PV, in particular, carries a higher risk of thrombosis, which may warrant the prescription of low-dose aspirin in many cases—unless contraindicated[13,14]. PV patients also require additional management strategies to ensure the early diagnosis of leukemia due to their increased risk[15].

With respect to the management of polycythemia, treatments focus on reducing the risk of complications and alleviating symptoms. The most common treatment for PV is phlebotomy[16]. This stimulates the bone marrow to produce new red blood cells, a process that requires iron and can reduce the body's iron stores, consequently leading to a long-term decrease in RBCs and serum iron. The main purpose of phlebotomy in PV patients is to reduce the risk of thrombosis[17]. An HCT threshold is typically established for management. Studies recommend maintaining HCT below 45% in the PV[18]. This low threshold for phlebotomy is due to the higher risk of thrombosis associated with PV, as well as research supporting this cut-off as more effective in reducing rate of cardiovascular death in PV patients[19,20]. On the other hand, in clinical practice, the aim is generally to keep HCT below 55% in secondary polycythemia[13]. However, patients with PV must be discouraged from donating blood, as they are permanently deferred from blood donation under WHO guidelines[21].

Blood donation is often driven by voluntary motives, symptoms relief or follows a diagnosis of secondary polycythemia made by a physician who attributes the blood results to an associated risk factor. Consequently, it is important to evaluate the theoretical risk that some individuals with presumed secondary polycythemia who frequently donate may, in fact, have undiagnosed PV.

Furthermore, there is a critical need for blood donations in conflict areas. The ongoing political conflict presents a significant challenge in providing adequate healthcare, including blood transfusions, to ensure a sufficient supply of safe blood for medical emergencies. Therefore, this study aimed to establish the prevalence and characterize the risk factors associated with primary and secondary polycythemia among Palestinian adult blood donors in the West Bank.

Methodology

Study design and setting

A cross-sectional study was conducted on adult Palestinian blood donors from September 2023–January 2024 in the blood donation center of a tertiary hospital, An-Najah National University Hospitals (NNUH).

Study population

The selection of blood donors at the center was based on regularly reviewed selection criteria in accordance with the guidelines from the Palestinian Ministry of Health and the World Health Organization (WHO) for assessing donor eligibility for blood donation[22]. The inclusion criteria included males aged 18–65 years who weighed more than 55 kg, had Hb concentrations above 14 g/dL, maintained a pulse rate of 60–100 beats per minute, and had a temperature not exceeding 37.5 °C. The exclusion criteria included participants with any of the following conditions: cardiovascular diseases such as ischemic heart disease, venous thrombosis, or thrombophlebitis; anemia, defined as a hemoglobin concentration of less than 14 g/dL in males; gastrointestinal diseases such as bleeding peptic or duodenal ulcers; metabolic and endocrine diseases such as insulin-dependent diabetes mellitus; chronic renal diseases; or malignant diseases.

Sample size and sampling technique

According to data from the West Bank's blood donation program published by the Ministry of Health in 2021, there were 30,730 blood donors[23]. With a 95% confidence interval, a 0.05 margin of error, and a 50% response distribution, the required sample size was 380. Convenience sampling was used. To reduce selection bias and consider the presence of risk factors and confounders, the sample size was increased to 760. A complete blood count (CBC) analysis was acquired from those with hemoglobin readings equal to or above 15 g/dL. Afterward, if CBC resulted in Hb concentrations above 16.5 g/dL or if the HCT was above 49%, the sample was referred for DNA sequencing to check for *JAK2* mutation.

Instruments and measures

Data collection was conducted through face-to-face interviews. The researchers developed a questionnaire on the basis entirely of a literature review. The questionnaire comprises four sections: Section 1: Demographic data, Section 2: Symptoms of polycythemia, Section 3: Risk factors for polycythemia, and Section 4: Blood donation-related information. The interviews clarified each section thoroughly to ensure confirmation and complete understanding. The demographic data described manual workers as those with a specific skill in a particular field. It excluded office workers, students, self-employed individuals, and government employees.

In terms of geography, mountains are defined as those 300 m above sea level[24]. In the symptoms section, the frequency terms are defined as follows: never (not once), seldom (at most once a week), sometimes (three to four times weekly), often (more than four times a week), and always (every day). The risk factors included substance use: cigarettes, categorized as mild (fewer than 10 cigarettes per day), moderate (10 to 19 cigarettes per day), and

heavy (20 or more cigarettes per day) smokers[25]. E-cigarette use was classified as mild (occasional), moderate (a limited number of times a day), or heavy (frequently throughout the day), and waterpipe use was categorized as mild (occasional), moderate (once daily), or heavy (more than once daily). Energy drinks and soft drinks were classified as mild (occasionally daily), moderate (1–3 cans daily), or heavy (more than four cans daily). Coffee was categorized as mild (occasionally daily), moderate (1–3 cups daily), or heavy (more than four cups daily). The alcohol classes included light (less than one glass per day), moderate (1 to 3 glasses per day), and heavy (more than 3 glasses daily). The participants were asked whether they had been exposed to factory smoke by living within 500 m of a factory or working in one. The questionnaire was evaluated by experts, revised after a pilot study, and then re-evaluated in a subsequent pilot study involving 30 participants.

Body weight in kilograms (kg) was measured with minimal clothing and without shoes on a calibrated digital scale to the nearest 0.1 kg. The standing height without shoes was measured in meters (m) with a wall meter nearest 1 mm (mm). Body mass index (BMI) was calculated by dividing weight in kilograms by the square of height in meters. The BMI categories were as follows: underweight (less than 18.5), normal weight (18.5–24.9), overweight (25.0–29.9), obese (30.0–34.9), and morbidly obese (35.0 and above).

Once a blood bank technician measured the donor's hemoglobin concentration with a hemoglobinometer, complete CBC analysis was acquired from those with hemoglobin readings equal to or above 15 g/dL for confirmation. Afterward, if CBC resulted in Hb concentrations above 16.5 g/dL or if the HCT was above 49%, the sample was referred for DNA sequencing to check for *JAK2* mutation.

Peripheral blood DNA was extracted via the NucleoSpin Blood Mini Kit (MACHEREY-NAGEL)[26] and quantified via a NanoDrop™ 2000 Spectrophotometer. A complete blood count (CBC) analysis was performed via an XN-1000 automated hematology analyzer (Sysmex Corporation) at An-Najah National University. The reference ranges were as follows: hematocrit (HCT) 42–54%, hemoglobin (Hb) 14–18 g/dL, white blood cell (WBC) count $4.5–10.0 \times 10^3$/μL, red blood cell (RBC) count $4.7–6.1 \times 10^6$/μL, mean corpuscular volume (MCV) 76–94 fL, and platelet count $140–450 \times 10^3$/μL. The *JAK2* p.V617F mutation was detected via PCR via the following primers: forward 5′-TCCTTAGTCTTTCTTTGAAGCAG-3′ and reverse 5′-ATTATAGTTTA CACTGACACCTAGC-3′. The PCR products were treated with ExoSAP-IT™ to remove unused primers and nucleotides. Cycle sequencing was performed via the BigDye™ Terminator v1.1 Cycle Sequencing Kit[27], followed by capillary electrophoresis on an ABI 3500 Genetic Analyzer. The detection threshold was 20%.

Diagnostic criteria

Polycythemia is defined as hemoglobin > 16.5 g/dL or hematocrit > 49% in men. PV is defined according to the latest update of the WHO classification of myeloproliferative neoplasms[28]. The *JAK2* mutation is detected in 96–99% of PV cases, with mutations occurring either in exon 14 (more commonly) or exon 12[5].

Statistical analysis

All data were analyzed using the IBM Statistical Package for the Social Sciences (SPSS) for Windows, version 27 (IBM Corp., Armonk, NY, USA). Continuous variables were reported as means ± standard deviations (SD) for normally distributed data and as medians with interquartile ranges (IQR) for non-normally distributed data. Categorical variables were presented as frequencies and percentages. The independent samples t-test was used for comparisons of continuous variables when normality assumptions were met. For categorical variables, Pearson's chi-square test or Fisher's exact test was employed as appropriate. A p-value of < 0.05 was considered statistically significant.

Univariable analyses were conducted to identify risk factors associated with secondary polycythemia. These factors were selected on the basis of a literature review, particularly in the Palestinian context. In the binary regression analysis, a p value of less than 0.25 from the univariable tests served as the threshold for including factors in subsequent analyses. This analysis explored the associations between secondary polycythemia (categorized as yes or no) and various factors that may influence the Hb concentration. The factors considered included body mass index, cigarette smoking, waterpipe smoking, e-cigarette use, energy drink consumption, coffee intake, geographic location, and occupation while controlling for age. The adjusted binary logistic regression model was used to assess the relative risk by calculating odds ratios (ORs) and 95% confidence intervals (CIs) for the identified risk factors. A p value of less than 0.05 was considered statistically significant.

Results

The study recruited 845 blood donors. Among these, 111 did not meet the inclusion criteria, 53 refused to participate, and 7 were excluded because of clotted EDTA blood samples, leading to a final sample size of 674 participants. The participants' ages ranged between 18 and 62 years, mostly between 18 and 35, with a mean age of 29.83 ± 8.8 years. Among them, 53.6% were from villages, 61.0% lived in mountainous areas (300–700 m above sea level), and 45.7% were workers. Moreover, 41.1% of the participants were overweight, 16.8% were obese, and 6.7% reported that they had been previously diagnosed with secondary polycythemia by a physician. The majority of the participants (80.9%) reported tobacco smoking in the last six months: 61.4% smoked cigarettes, 8.2% smoked E-cigarettes, and 29.2% smoked waterpipes. In particular, 67.1% of the cigarette smokers were heavy smokers. Moreover, 18.8% of the participants were dual smokers; 11.7% smoked both cigarettes and waterpipes; 4.0% smoked cigarettes and e-cigarettes; and 3.11% smoked waterpipes and e-cigarettes. The other substances used were energy drinks (47.5%), coffee (79.5%), and illicit drugs (0.7%) (Table 1).

Supplementary File 1 describes symptoms related to polycythemia among participants ($n = 674$): 17.8% of participants reported having headache sometimes, 16.5% reported experiencing fatigue and exhaustion without physical activity sometimes, and 11.7% reported having early satiety sometimes. Other symptoms, including skin redness and skin itching after a shower, were sometimes experienced by 7.3% and 6.8% of the participants,

		n(%)
BMI	Underweight	4(0.6)
	Normal	228(33.8)
	Overweight	277(41.1)
	Obese	113(16.8)
	Morbidly obese	52(7.7)
Residency	City	252(37.4)
	Village	361(53.6)
	Camp	61(9.1)
Geography	Mountain	411(61.0)
	Plain	240(35.6)
	Valley	23(3.4)
Occupation	Manual workers	308(45.7)
	Students	66(9.8)
	Employees in various occupations	188(27.9)
	Self-employed	100(14.8)
	Unemployed	12(1.8)
Tobacco and other substance use	Cigarette smoking	414(61.4)
	E-Cigarette smoking	55(8.2)
	Waterpipe smoking	197(29.2)
	Energy drinks use	320(47.5)
	Coffee use	535(79.5)
	Soft drink use	517(76.7)
	Alcohol use	16(2.4)
	Cigarettes and waterpipe	79(11.7)
	Cigarettes and E-cigarettes	27(4.0)
	Waterpipe and E-cigarettes	21(3.11)
	Illicit drugs	5(0.7)
	Exposure to factories' smokes	153(22.7)
	Quarries (500 m)	30(4.5)

Table 1. Sociodemographic data ($n = 674$).

respectively. Only 4.2% reported respiratory diseases, 2.8% used inhalers or nebulizers regularly, and 0.3% used androgenic steroids (Supplementary File 1.

Supplementary File 2 presents information related to blood donation ($n = 674$). The blood donation portion of the survey revealed that 23.1% of the participants donated more than once a year; 75% of these donors donated twice a year, with a 6-month gap between each donation. The data indicated that 12.8% of the participants experienced symptoms, including weakness, headaches, and dizziness, which led them to donate, with 96.3% reporting an improvement in their symptoms after donating blood. Additionally, 85.2% of the participants donated voluntarily (Supplementary File 2.

CBC results for participants ($n = 410$)

Among the 674 participants, 410 had Hb concentrations > 15 g/dL and underwent CBC tests for confirmation. Among the 410 patients, 112 (16.6%) had polycythemia (110 with Hb > 16.5 g/dL and 2 with HCT > 49%) and were therefore sent for DNA sequencing. The mean Hb and HCT values for participants with secondary polycythemia and those who underwent DNA sequencing were 17.24 ± 0.73 and 49.82 ± 2.40, respectively. Furthermore, only 3.6% of polycythemia patients had HCT levels above 55%, suggesting that most were below the donation threshold and considered well controlled (Table 2).

Univariable analysis of risk factors for secondary polycythemia

One hundred and ten of the 112 participants who underwent DNA testing completed the questionnaire. Among them, 12.7% were moderate cigarette smokers, 42.7% were heavy cigarette smokers, and 16.4% were heavy waterpipe smokers. Waterpipe smoking was significantly associated with polycythemia ($p = 0.023$). With respect to other consumption habits, 28.2% were heavy coffee drinkers, 30.0% consumed mild energy drinks, and 37.6% and 28.4% consumed mild and moderate soft drinks, respectively (Table 3).

Adjusted binary logistic regression for risk factors associated with increased risk of secondary polycythemia

The risk factors associated with an increased risk of secondary polycythemia were moderate smoking (OR 2.223, 95% CI 1.041–4.746, $p = 0.039$), heavy cigarette smoking (OR 2.301, 95% CI 1.246–4.249, $p = 0.008$), and heavy

		Mean ± SD		
	Minimum–maximum	Polycythemia (Yes) $n=112$	Polycythemia (No) $n=298$	*P* value
Hb (g/dL)	16–21.4	17.24 ± 0.73	15.45 ± 0.89	< 0.001
HCT (%)	46.6–61.3	49.82 ± 2.40	45.07 ± 2.89	< 0.001
RBC (10^6/uL)	5–7.36	5.87 ± 0.38	5.36 ± 0.39	< 0.001
MCV (fL)	73.5–92.4	84.84 ± 3.21	84.49 ± 4.08	0.364
WBC (10^3/uL)	3.08–14.66	7.83 ± 2.18	7.34 ± 2.05	0.037
Platelets (10^3/uL)	71–438	230.97 ± 59.96	237.91 ± 57.64	0.226

Table 2. CBC results ($n=410$).

		Polycythemia ($n=110$)*	No Polycythemia ($n=300$)	
		n(%)	*n*(%)	*P* value
Cigarette smoking	Mild	8(7.3)	22(7.3)	0.997
	Moderate	14(12.7)	38(12.7)	
	Heavy	47(42.7)	125(41.7)	
	None smoker	41(37.3)	115(38.3)	
Waterpipe smoking	Mild	14(12.7)	36(12.0)	0.023
	Moderate	6(5.5)	23(7.7)	
	Heavy	18(16.4)	20(6.7)	
	None smoker	72(65.5)	221(73.7)	
Energy drink intake	Mild	33(30.0)	98(32.7)	0.338
	Moderate	13(11.8)	42(14.0)	
	Heavy	3(2.7)	2(0.7)	
	None user	61(55.5)	158(52.7)	
Coffee intake	Mild	15(13.6)	46(15.3)	0.250
	Moderate	36(32.7)	91(30.3)	
	Heavy	31(28.2)	109(36.3)	
	None user	28(25.5)	54(18)	
Soft drinks	Mild	41(37.6)	110(36.7)	0.113
	Moderate	31(28.4)	116(38.7)	
	Heavy	0(0.0)	2(0.7)	
	None user	37(33.9)	72(24)	
E-cigarette smoking	Mild	4(3.6)	18(6.0)	0.300
	Moderate	2(1.8)	1(0.3)	
	Heavy	3(2.7)	6(2.0)	
	None smoker	101(91.8)	275(91.7)	
Alcohol	Light	2(1.8)	5(1.7)	1.000
	Moderate	0(0.0)	0(0.0)	
	Heavy	0(0.0)	2(0.7)	
	None user	108(98.2)	293(97.7)	
Factories', Cars' emissions	Yes	20(18.2)	75(25.0)	
Quarries (500 m)	Yes	4(3.6)	16(5.3)	
Respiratory diseases	Yes	5(4.5)	14(4.7)	
Inhaler use	Yes	3(2.7)	5(1.7)	
Geography	Mountain	70(63.6)	185(61.7)	0.927
	Plain	37(33.6)	107(35.7)	
	Valley	3(2.7)	8(2.7)	
Occupation	Manual workers	45(40.9)	134(44.7)	0.721
	Students	11(10)	32(10.7)	
	Employees in various occupations	32(29.1)	84(28)	
	Self-employed	21(19.1)	43(14.3)	
	Unemployed	1(0.9)	7(2.3)	

Table 3. Univariate analysis of risk factors for secondary polycythemia ($n=410$). *Out of the 112 participants with polycythemia, two did not complete the questionnaire data on key variables, especially smoking and dietary intake. Therefore, only 110 participants were included in this table.

Secondary polycythemia		OR	95% CI		*P* value
			Lower	Upper	
BMI		1.014	0.972	1.058	0.523
Age		1.020	0.992	1.048	0.168
Cigarette smoking	Mild	1.245	0.522	2.970	0.621
	Moderate	2.223	1.041	4.746	**0.039**
	Heavy	2.301	1.246	4.249	**0.008**
	None smoker	1			
E-cig smoking	Mild	0.760	0.256	2.256	0.621
	Moderate	1.607	0.274	9.424	0.599
	Heavy	1.378	0.343	5.530	0.651
	None smoker	1			
Waterpipe smoking	Mild	0.955	0.500	1.823	0.889
	Moderate	1.072	0.409	2.810	0.887
	Heavy	5.019	2.364	10.653	**< 0.001**
	None smoker	1			
Energy drinks	Mild	0.923	0.566	1.503	0.747
	Moderate	0.770	0.374	1.583	0.477
	Heavy	3.570	0.770	16.562	0.104
	None user	1			
Coffee	Mild	0.766	0.370	1.586	0.473
	Moderate	0.617	0.342	1.114	0.109
	Heavy	0.444	0.234	0.843	**0.013**
	None user	1			
Geography	Mountainous area	1.168	0.326	4.182	0.811
	Plain	1.044	0.285	3.821	0.948
	Valley	1			
Occupation	Manual worker	1.516	0.183	12.583	0.700
	Student	2.760	0.308	24.691	0.364
	Employee	1.879	0.223	15.795	0.562
	Self-employed	2.111	0.245	18.198	0.497
	Not working	1			

Table 4. Adjusted binary logistic regression for risk factors associated with secondary polycythemia ($n = 674$). Significant values are in [bold].

waterpipe smoking (OR 5.019, 95% CI 2.364–10.653], $p \leq 0.001$). Heavy coffee use was inversely related to secondary polycythemia (OR 0.444, 95% CI 0.234–0.843, $p = 0.013$) (Table 4).

DNA sequencing results

The 112 blood samples that matched the polycythemia diagnostic values of Hb or HCT were sent for DNA sequencing at the NNUH laboratory. The reverse primer was used to detect the mutation in 103 samples, whereas the forward primer was used in 9 samples. The results revealed that none of the patients had the *JAK2* p.V617F exon 14 mutation.

Discussion

This study aimed to determine the prevalence of polycythemia and identify its risk factors among adult Palestinian blood donors in the West Bank from September 2023 to January 2024. The study highlights the prevalence of polycythemia among blood donors, with 16.6% affected. Furthermore, the absence of the JAK2 p.V617F mutation among participants with polycythemia suggests that secondary polycythemia is more prevalent in this population. A significant number of those diagnosed were heavy smokers, with a particularly high prevalence among both cigarette and waterpipe users. This finding points to a strong link between tobacco use and the development of polycythemia, further supporting the well-established evidence that smoking is a risk factor for this condition. Interestingly, heavy coffee consumption appeared to have a protective effect against polycythemia, indicating that dietary factors might play a role in managing or reducing the risk of this blood disorder. These findings suggest that integrating lifestyle modifications, such as reducing smoking and modifying coffee consumption habits, may be beneficial strategies for lowering the risk of developing polycythemia. Overall, these results emphasize the need for public health initiatives aimed at promoting healthier lifestyle choices to combat the risk of polycythemia.

Diagnosing polycythemia, especially its secondary forms, can be challenging[29]. Hypoxia-induced polycythemia is related to multiple causes, including physiological erythrocytosis in people living at high

altitudes and cardiopulmonary pathologies, such as chronic obstructive pulmonary disease and smoking[29]. Polycythemia secondary to smoking represents one of the most common secondary causes that trigger systemic hypoxia, leading to increased production of erythropoietin by the kidneys[29]. In agreement with other studies, the findings of this study revealed that most blood donors were tobacco smokers[30]. Moreover, the presence of polycythemia in this study was associated with the pattern of tobacco smoking. It was previously thought that waterpipes are not associated with polycythemia because they are typically used less frequently than cigarettes[29,31]. The results of this study indicated that heavy waterpipe smokers (those who smoked more than once daily) were five times more likely to develop polycythemia than nonsmokers were, whereas moderate and heavy cigarette smokers (> 10 cigarettes daily) were twice as likely to develop polycythemia. This can be explained by the hypoxia caused by the profound increase in carboxyhemoglobin levels associated with waterpipe smoking compared with cigarette smoking[32]. This concept is supported by previous studies that reported similar results concerning waterpipe smoking and elevated hemoglobin concentrations[29,33,34]. Waterpipe smoking is common among young people in general and Palestinians in particular, yet few studies address its effects on health[35]. There is an emergent need for regulatory measures to curb this rapidly growing epidemic[35]. Over the past ten years, there has been an increase in waterpipe smoking among Palestinians. Local studies have indicated that waterpipe smoking is more common among males, younger individuals, and those with lower education levels, often as a substitute for cigarette smoking and often starting at an earlier age (< 18)[30,36–38]. Moreover, some of these studies linked their use with obesity and eating behaviors. In light of the limited surveillance of waterpipe smoking and polycythemia in the region and considering the growing popularity of waterpipe smoking in the Middle East and among Palestinians in particular[30], these results raise new global concerns about the effects of waterpipe smoking on secondary polycythemia. Therefore, we recommend considering waterpipe smoking history in medical records. Additionally, it is important to raise awareness among the population about the adverse health impacts of waterpipe smoking.

Tobacco smoking is highly prevalent among Palestinians on the West Bank, according to the Palestinian Central Bureau of Statistics (40.1%)[39]. A recent study revealed that cigarette smoking is the most popular method of tobacco use (67.5%), with 53.9% of smokers reporting smoking more than 20 cigarettes a day for a median duration of 7 years. A highly concerning point is the early age of initiation, as 69.4% of smokers are young adults (20–40 years old)[30]. The high prevalence of tobacco smoking underscores the public health challenge posed by tobacco use in the West Bank. Therefore, we recommend promoting early cessation and increasing awareness of its adverse consequences. More studies are needed in this field.

The results indicated that secondary polycythemia is associated with tobacco smoking being a major risk factor that poses significant health risks, particularly to the cardiovascular system[40]. Nonetheless, the question of whether secondary polycythemia serves as an independent risk factor for these conditions remains a subject of debate. A notable example is the relationship between thromboembolic events and polycythemia. It is well-documented that polycythemia vera (PV) is associated with an increased incidence of thrombotic events. In contrast, the current literature indicates that secondary polycythemia may not independently increase the risk of thromboembolic complications[41,42]. However, the existing body of research addressing this issue is limited, highlighting the need for further investigations to elucidate the nature of this relationship.

In agreement with other studies, the findings of this study suggested that coffee may have a modest protective effect on polycythemia. This finding is consistent with a previous study, which attributed the protective effect of coffee to its anti-inflammatory and antitumor immune response[43]. However, as studies elucidating the underlying mechanism are lacking, further research is needed to explore this relationship. In addition, the study did not find any significant associations between e-cigarette smoking or energy drink intake and secondary polycythemia. The lack of association with e-cigarettes may be due to their low popularity in the study population and their emerging status as a potential hazard in Palestine[44]. Although energy drinks are known to contain caffeine, the study did not establish a significant correlation between polycythemia and the consumption of energy drinks. Further research is needed to explore the effects of energy drinks on blood components, considering their widespread popularity and the present scarcity of scientific literature on the topic.

Given that the study population consisted primarily of healthy young blood donors, the prevalence of common risk factors for secondary polycythemia, such as respiratory diseases and regular inhaler use, was notably low, reported at 4.2% and 2.8%, respectively. Although a significant percentage of participants, 61.0%, resided in mountainous areas (300–700 m above sea level), this geographic factor did not yield significant results in our analysis. Previous studies have suggested that substantial altitude-related hematological changes typically emerge at elevations exceeding 2500 m. This may explain why altitude was not a significant risk factor in our study, as the elevations experienced by our participants were insufficient to induce these changes[45].

The only other study regarding PV conducted in Palestine was a case–control study. The frequency of the *JAK2* p.V617F mutation was reported in three study groups: (69.2%) in group 1, which included Hb concentrations > 18.5 g/dL for men and > 16.5 g/dL for women; (51.6%) in group 2, which included males with Hb concentrations between 16.6 g/dL and 18.5 g/dL but did not meet the WHO criteria; and (21.7%) in the control group[9]. They also studied the *JAK2* exon 12 mutation and reported that 100% of the samples were wild-type[9]. Compared with this study, we included a wider range of Hb (16 g/dL–21.4 g/dL) from our participants; however, zero *JAK2* p.V617F mutation cases were reported. We used a technique with a 20% detection threshold, whereas they used ARMS PCR with a detection threshold of approximately 1%. Consequently, we may have overlooked low-frequency mutations. Additionally, the age range in the cited study spanned from 18 to 83 years, whereas our study covered individuals aged 18–62 years. This difference indicates that they included older patients, potentially influencing the JAK2 p.V617F mutation results, given that the median age for a polycythemia vera diagnosis is in the sixties[46,47].

Studies among patients diagnosed with PV in neighboring Arab populations have shown that the *JAK2* p.V617F exon 14 mutation is common, with frequencies of 91% in Sudanese, 91% in Saudi Arabian, and 81.4%

in Egyptian PV patients[10,48,49]. They applied ACB-PCR with a detection threshold below 0.1%, ABI Prism at approximately 20%, and ARMS PCR near 1%. These variations in methodologies contrast with this study, which had a detection threshold of 20%, potentially impacting the results. Importantly, the other studies focused primarily on patients with PV, as there are few investigations in the Arabic region regarding polycythemia in a generally healthy population such as ours. Additionally, this study did not include *JAK2* exon 12 testing because it is less common than the mutation in exon 14[5]. The previously mentioned Palestinian study further confirmed this finding[9]. However, further studies covering both mutations would help confirm these results.

An HCT threshold is typically established for the management of polycythemia. While many studies recommend maintaining HCT below 45% in the PV, in clinical practice, the threshold is generally considered to be less than 55% in secondary polycythemia[13,18]. In this study, 3.6% of secondary polycythemia cases had HCTs above this threshold, indicating that most cases were well controlled in this regard. It is also worth mentioning that these patients, once PV has been ruled out, are generally safely eligible to donate blood, which may eliminate the need for therapeutic phlebotomy.

Conflict areas such as Palestine often face a critical need for blood donation. Approximately one-third of people with secondary polycythemia donate blood more than once a year. Among those who experienced symptoms before donation, 96.3% reported an improvement after donation. This subjective relief of symptoms may explain why people are motivated to donate blood frequently.

Several factors limited this study, primarily stemming from its observational cross-sectional design, which may introduce information bias and does not allow for the establishment of causality. The samples were not tested for the JAK2 exon 12 mutation. The high detection threshold of the JAK2 mutation analysis may have resulted in the missed identification of low-frequency mutations, potentially leading to false-negative results for JAK2 mutations. Additionally, the study faced a limitation due to the exclusion of female blood donors, prompted by a lack of eligible women willing to participate. This exclusion resulted in a solely male participant pool, introducing selection bias. While most research indicates that males are more likely to have polycythemia vera (PV), one systematic review and meta-analysis revealed no statistically significant difference in the annual incidence of PV between sexes[50]. Furthermore, the exclusion of participants with a history of cardiovascular diseases, including ischemic heart disease, venous thrombosis, or thrombophlebitis, may have led to the omission of essential cases of secondary polycythemia or PV that might be linked to these conditions. Although the questionnaire included questions on chronic respiratory diseases, it lacked specificity regarding sleep apnea, an important risk factor that may have been overlooked. Moreover, the debilitating nature of many chronic respiratory diseases often makes blood donation unfeasible for these patients, which explains the underrepresentation of this group among donors. Finally, while the majority of blood donors reported adhering to the standard recommendation of a 6-month interval between donations, a subset donated more frequently (every 3–4 months). This raises concerns about potential iron depletion due to more frequent donations, which could sometimes contribute to the underestimation of polycythemia. Moreover, the study did not measure erythropoietin levels in the samples, which could have provided further insight into the underlying causes of polycythemia. Despite these limitations, this study is a significant contribution to the field, as it is the first study in Palestine to investigate the risk factors and genetics for polycythemia among Palestinians. Additionally, this study contributes to the body of evidence regarding the impact of waterpipe smoking on polycythemia and the correlation between patterns of tobacco smoking and polycythemia.

Conclusion

The research findings indicated no occurrence of *JAK2* p.V617F exon 14 mutation-associated PV among Palestinian blood donors. However, the results indicated a notable prevalence of secondary polycythemia. This study explored the risk factors associated with secondary polycythemia, revealing that individuals who were moderate to heavy cigarette smokers, as well as heavy waterpipe smokers, had a significantly heightened risk of developing this condition. These findings contribute to the existing evidence on the effects of waterpipe smoking on polycythemia and the relationship between tobacco smoking patterns and polycythemia. Moreover, these significant findings underscore the critical need for comprehensive public health interventions aimed at promoting smoking cessation and increasing awareness of the potential health risks linked to specific lifestyle habits.

Recommendations

Future studies may include both JAK2 p.V617F and JAK2 exon 12 mutations, a wider range of adult participants, particularly those in their sixties and above, and higher hemoglobin values, which may result in more precise and representative results regarding the prevalence of PV.

Data availability

The authors confirm that all data generated or analyzed during this study are included in this manuscript.

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Author contributions
BD: Study conception and design, Data collection and curation, Formal analysis. MA, GO, RH, AS: Study conception and design, collaborated in formulating the idea, and data collection. BD, RZ , SE, SB, and MD: Interpreting the results. BD, MA, GO, RH, and AS writing the original draft. All the authors approved the final version for publication and agreed to take responsibility for all aspects of the work, as well as to thoroughly investigate and resolve any questions regarding the accuracy or integrity of any part of the work.

Declarations

Competing interests
The authors declare no competing interests.

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